Bovine embryos cultured *in vitro* in the presence of antioxidants: implications for blastocysts development, quality and cryotolerance

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Introduction

The increase in intracellular reactive oxygen species (ROS) due to the high oxygen tension during *in vitro* culture (IVC) induces oxidative stress, leading to apoptosis and embryonic developmental failure (1). Addition of antioxidants during IVC appears to increase the resistance of bovine embryos to the oxidative stress and consequently improves cryotolerance (2). Thus, the aim of this study was to evaluate the effects of intracellular (cysteine and β-mercaptoethanol) and extracellular antioxidants (catalase) during IVC on the embryo development and cryoresistance, as well as the amounts of intracellular ROS and the percentage of blastomeres undergoing apoptosis.

Materials and Methods

Oocytes (n=733) were matured and fertilized *in vitro* for 24 h. Presumptive zygotes were IVC during the first 72 h (up to day 3) in SOF with 0.6 mM cysteine (CIST), 100 µM β-mercaptoethanol (βME), 100 UI catalase (CAT) or without antioxidants (Contr). From day 3 to the end (day 7), all embryos were cultured in SOF medium. All cultures were conducted at 38.5°C in 5% CO_2 in air. The cleavage and blastocysts rates were evaluated, respectively, at 72 and 168 hours post-insemination, when the blastocysts were vitrified (n=151; Ingámed®, Maringá-PR, Brasil), stained (n=45) with 5 µM of the fluorescent probe H_2DCFDA or stained (n=71) for TUNEL according to the technique (3). The embryos vitrified were thawed and cultured for 24 h to evaluate the re-expansion rates. Embryos stained with H_2DCFDA and TUNEL were evaluated under an epifluorescence inverted microscope (excitation 495/510-550nm and emission 520/590 nm, respectively) and the levels of intracellular ROS (arbitrary fluorescence units) were measured by Q-Capture Pro image software. The cleavage, embryo development, levels of ROS and percentage of apoptosis were analyzed by ANOVA followed by Tukey's test, and re-expansion rates by Chi-square test ($P<0.05$). Data are presented as Mean ± SEM.

Results and Discussion

Results are summarized in Table 1. The fluorescence intensity were lower in CIST and CAT compared to the Control ($P<0.05$). The percentage of apoptotic cells was reduced in CIST compared to the Control ($P<0.05$). Then, data showed that the reduction in the levels of intracellular ROS resulted in a decrease in the incidence of apoptosis in IVP bovine embryos. In conclusion, addition of cysteine or catalase during IVC improves the quality of bovine embryos measured by the intracellular levels of ROS and rates of apoptosis, however did not affect the embryo survival after vitrification.

Table 1. Cleavage, development to blastocyst, embryo survival after vitrification, measurement of intracellular ROS (Fluorescent intensity) and the incidence of apoptotic cells in bovine embryos cultured in medium supplemented with antioxidants

Groups	Cleavage *	Blastocysts *	Re-expansion 24h † (%)	Arbitrary Fluorescence Units *	Number of blastomeres *	Apoptotic cells *
Control	82.9 ± 3.2	48.7 ± 3.4	76.0	1.0 ± 0.07^{a}	85.7 ± 4.6	4.32 ± 1.2^{a}
CIST	80.5 ± 3.1	37.1 ± 3.2	71.0	0.6 ± 0.06^{c}	81.9 ± 3.3	1.96 ± 0.4^{b}
βME	79.6 ± 3.0	33.4 ± 9.4	73.3	0.9 ± 0.05^{ab}	80.5 ± 4.8	2.17 ± 0.6^{ab}
CAT	83.6 ± 3.7	53.0 ± 7.1	83.6	0.7 ± 0.06^{bc}	90.9 ± 4.1	2.05 ± 0.5^{ab}

*Means followed by different letters in the same collum differ ($P < 0.05$) by Tukey´s test. † Means followed by different letters in the same collum differ ($P < 0.05$) by Chi-square.

References:

(1) Guérin P et al. 2001. Human Reproduction Uptade, 7:175-189; (2) Hosseini SM et al. 2009. J Assist Reprod, 6:355-364; (3) Paula-Lopes et al. 2002. Biology of Reproduction, 66:1169-1177.

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